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METHOD AND APPARATUS FOR DIRECT MEASUREMENT OF HEMOGLOBIN SPECIES IN WHOLE BLOOD

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FIELD OF THE INVENTION

The invention relates to determination of hemoglobin species in whole, undiluted blood.

BACKGROUND OF THE INVENTION

Many known apparatus exist for the photometric analysis of blood to determine its constituent components. Some apparatus, including those disclosed in U.S. Patent Nos. 4,134,678 and 4,324,556 use the Beer-Lambert Law of absorption spectroscopy which defines the photometric relationships used in measuring the concentration of a colored compound. In general, under the Beer-Lambert Law, optical density or absorbance of a colored liquid is directly proportional to the concentration of the liquid and the path length of light passing through the liquid.

However, it has been observed (N. M. Anderson, et al., "Light-Absorbing and Scattering Properties of Nonhemolyzed Blood," Phys. Med. Biol., Vol. 12, pp. 173-184, 1967) that whole blood does not obey the Beer-Lambert Law. In particular, the optical density or absorbance of whole blood is proportional neither to the path length nor the concentration of hemoglobin (the primary coloring component of blood). This discrepancy has been attributed to the scattering of light or, more generally, the scattering of the measuring radiation. The scattering of radiation arises from the discontinuity in the index of refraction at the red cell-plasma interface. In addition, radiation that is scattered once will likely be scattered again by other cells.

Figures 1 and 2 demonstrate the effects of radiation scattering in whole blood using a conventional optical geometry (for example, that found in a Waters Instrument, Oxicom oximeter). Referring to Fig. 1, light-emitting

diode 6 emits monochromatic light having a wavelength of approximately 660 nanometers. Light passes through cuvette 7 having an absorbance path, t, on the order of 1.2 millimeters. Photodetector 8 is placed a distance, d_1, approximately 5 millimeters from cuvette 7 to detect light passing through cuvette 7. Detector 8 has a detecting area of approximately 5.1 square millimeters. With the conventional optical geometry of Fig. 1, a substantial amount of light, represented by region 9, is lost due to scattering, thereby greatly affecting the measurement accuracy of the absorbance or optical density of blood contained within cuvette 7.

Referring now to Fig. 2, the optical density of whole blood measured using the conventional optics of Fig. 1, is shown as a function of hemoglobin concentration, using a common light measuring wavelength of 660 nanometers. As can be seen, the optical density of whole blood is a nonlinear function of hemoglobin concentration, rather than the linear function predicted by the Beer-Lambert Law. This deviation is due primarily to the effects of light scattering.

Prior art photometric blood analysis devices often avoid the deleterious effects of light scattering in whole blood by hemolyzing and/or diluting the blood sample before a photometric measurement. Such hemolysis and dilution requires additional apparatus such as diluent containers, pumps, conduits, ultrasonic generators, freezing units and the like. As a result, apparatus which employ hemolysis and dilution of blood samples are often bulky and expensive, and are typically not portable and are relegated to laboratory use only.

SUMMARY OF THE INVENTION

The present invention avoids the above-noted problems of known hemoglobinometers and oximeters by employing a unique optical configuration which greatly reduces the effects of radiation scattering by whole blood, and allows the Beer-Lambert Law to more accurately describe the concentrations of various hemoglobin species.

The present invention allows the measurement of the concentrations of four or more species of hemoglobin in whole, undiluted blood. These species include, for example, oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, methemoglobin, and sulfhemoglobin.

The present invention involves irradiating a sample of whole, undiluted blood with selected wavelengths of radiation chosen from regions of the hemoglobin absorbance spectrum where radiation absorbance is much greater than radiation scattering. The wavelengths are also chosen to maximize the measurement accuracy in distinguishing one hemoglobin species from another, and to minimize the sensitivity of the molar extinction coefficients of the individual hemoglobin species under consideration due to small changes in radiation wavelength. The invention also uses a short absorption path by employing a thin blood sample cuvette, chosen to minimize total radiation scattering relative to absorbance by reducing the chance that a photon will be scattered by more than one red blood cell.

In addition, a relatively large area radiation detector is used in order to capture radiation scattered at wide angles by the whole blood sample. The light detector is placed close to the cuvette thereby increasing the receiving aperture half angle of the detector and minimizing light losses caused by radiation scattered at large angles.

Thus, the optical configuration of the present invention maximizes the optical absorbance of whole, undiluted blood and minimizes the effects of light scattering so that the apparent optical density of the sample measured by the detector is due primarily to absorbance with as small a contribution by light scattering as possible.

The optical density of the whole, undiluted blood sample is determined at each of the measuring radiation wavelengths, and, using predetermined molar extinction coefficients for each of the desired hemoglobin species at each of the measuring radiation wavelengths, a set of simultaneous equations can be solved to calculate the concentration of each desired hemoglobin species.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic representation of the optics of a known oximeter.

Fig. 2 is a graph of the optical density of whole blood as a function of hemoglobin concentration for the optical configuration of Fig. 1.

Fig. 3 is the optical configuration of the present invention.

Fig. 4 is a graph of the optical density of four hemoglobin species as a function of measuring radiation wavelength.

Fig. 5 is a graph of the optical density of whole blood measured using the optical configuration of Fig. 3 as a function of hemoglobin concentration for various measuring radiation wavelengths.

DETAILED DESCRIPTION OF THE INVENTION

Referring to Fig. 3, the invention includes a selectable radiation source 10, which is preferably a visible light radiation source, a thin cuvette 11, used to hold a sample of whole blood, a large area radiation detector 12, and a controlling and calculating unit 13 used to control radiation source 10 and to solve a set of simultaneous equations using the Beer-Lambert Law and the radiation intensities detected by radiation detector 12.

Controlling and calculating unit 13 can be, for example, an appropriately programmed digital computer such as that in U.S. Patent No. 4,134,678, the disclosure of which is expressly incorporated herein by reference.

Radiation source 10 provides radiation of a plurality of wavelengths for application to cuvette 11. According to the preferred embodiment, selectable radiation source 10 emits visible radiation at a plurality of selected frequencies. These frequencies are selected using a number of criteria. First, the frequencies are selected from regions of the absorbance spectrum of hemoglobin where radiation absorbance is much greater than radiation scattering. In addition, the specific radiation frequencies are chosen to maximize measurement accuracy in distinguishing one hemoglobin species from another and to minimize the sensitivity of the molar extinction coefficients to small variations in the radiation wavelength emitted by selectable radiation source 10. Finally, the radiation wavelengths are chosen so that for each wavelength, the molar extinction coefficient for at least one of the hemoglobin species under consideration is very large.

In the preferred embodiment, when measuring the four hemoglobin species oxyhemoglobin, deoxyhemoglobin, carboxyhemoglobin, and methemoglobin, the four visible

light wavelengths 520, 560, 582, and 598 nanometers have proven to produce good measurement accuracy and are preferred. Other sets of measuring wavelengths that have proven successful include: 506, 569, 577 and 585 nanometers; 506, 560, 569 and 585 nanometers; 510, 542, 560 and 577 nanometers; and 535, 585, 595 and 627 nanometers. Specification of these sets of four wavelengths should not be considered a limitation on the present invention, and it should be understood that other sets of wavelengths may also be acceptable. In general, the number of radiation wavelengths is equal to the number of hemoglobin species to be detected. Thus when concentration of sulfhemoglobin is also to be measured, a set of five measuring wavelengths is chosen. The preferred set of five wavelengths is 520, 560, 582, 598 and 620 nanometers. Thus the wavelength of 620 nanometers is added to the above-noted preferred set of four measuring wavelengths. The measuring wavelength of 620 nanometers can also be added to the other sets of four wavelengths to produce acceptable sets of five measuring wavelengths.

Once the specific wavelengths are chosen, appropriate molar extinction coefficients are measured or calculated in a known manner for each of the hemoglobin species to be detected, at each of the measuring radiation wavelengths. These are then stored in controlling and calculating unit 13 for use in solving the simultaneous equations.

Selectable radiation source 10 may be constructed of various components. For example, source 10 may be a white light source in combination with interference filters selected to pass only the selected radiation frequencies or a white light source in combination with a controllable monochromator or a controllable diffraction grating which cooperate to produce the selected measuring radiation frequencies. In addition, selectable source 10 could be a

tunable laser which is tuned under control of controlling and calculating unit 13. Selectable radiation source 10 could also be constructed of light-emitting diodes with appropriate interference filters cooperating to generate and pass the desired measuring radiation frequencies.

Thin cuvette 11 is preferably of glass or other material which is substantially transparent to the selected measuring radiation frequencies, and can be a capillary tube. Cuvette 11 is constructed so that the absorbance path or depth of a whole undiluted blood sample contained within cuvette 11 minimizes light scattering. Absorbance paths on the order of between 80 and 150 micrometers have proven successful.

Large area radiation detector 12 is sensitive to the selected measuring radiation frequencies. The detecting area of detector 12 and the distance, d_2, between detector 12 and cuvette 11 are chosen to capture radiation scattered at wide angles by the whole undiluted blood sample contained within cuvette 11. Detector 12 can be, for example, a type SD-1100-11-21-181 detector available from Silicon Devices. This preferred detector has a detecting area on the order of about 600 square millimeters, and is placed a distance, d_2, within the range of 2 to 10 millimeters from cuvette 11. This configuration results in an aperture half angle, , of radiation emanating from cuvette 11 and impinging upon detector 12 of at least approximately 70°.

In operation, controlling and calculating unit 13 controls selectable radiation source 10 to produce one of the selected measuring radiation frequencies. Controlling and calculating unit 13 then senses the output of detector 12 and compares it with the intensity of radiation incident upon cuvette 11, to determine the optical density of the whole undiluted blood sample within cuvette 11 at the selected radiation frequency. Then, using the molar

extinction coefficients at the selected radiation frequency for each of the constituent components of unknown concentration within the whole blood sample, a first simultaneous equation is created. Controlling and calculating unit 13 then causes selectable radiation source 10 to emit another of the selected measuring radiation frequencies, and another simultaneous equation is created using the molar coefficients of extinction for each constituent component of unknown concentration, at the newly selected frequency. This process is repeated until the number of simultaneous equations within controlling and calculating unit 13 is equal to the number of constituent components of unknown concentration. These simultaneous equations are then solved by controlling and calculating unit 13 to produce the concentrations of the desired constituent components of the whole undiluted blood sample contained within cuvette 11.

The molar extinction coefficients at each of the selected frequencies for each of the constituent components being measured can be determined empirically using the graph of Fig. 4. Fig. 4 is a graph of the optical density of four different hemoglobin species, oxyhemoglobin (O2), carboxyhemoglobin (CO), methemoglobin (Met) and deoxyhemoglobin (RHb), of known concentrations, as a function of measuring radiation wavelength. From the graph of Fig. 4, molar extinction coefficients for each hemoglobin species can be calculated in a known manner from the detected optical density at any radiation wavelength.

Referring now to Fig. 5, the optical density of a whole, undulated blood sample using the optical configuration of Fig. 3 is shown as a function of hemoglobin concentration at three measuring wavelengths, 506, 560 and 569 nanometers. A substantially linear relationship exits between varying hemoglobin

concentration and optical density at each of the selected measuring radiation wavelengths, in contrast to the highly nonlinear relationship illustrated in the graph of Fig. 2 using the conventional optics of Fig. 1.

Although the present invention has been described in connection with a single preferred embodiment, it will be understood that this preferred embodiment is by way of example only, and should not be considered a limitation on the present invention.